Kidney International, Vol. 15 (1979), pp. 160–166

Pharmacokinetics of gentamicin in the treatment of renal infection: A therapeutic anomaly explained

THOMAS MILLER, SUSAN PHILLIPS, and DEREK NORTH

Department of Medicine, Auckland Hospital, Auckland, New Zealand

Pharmacokinetics of gentamicin in the treatment of renal infection: A therapeutic anomaly explained. A model of pyelonephritis was used to investigate the ability of gentamicin to eradicate experimental renal infection. *Escherichia coli* pyelonephritis was induced in 56 rats, and treatment with gentamicin was started 7 days after challenge and was maintained for 11 days. Renal tissue examined at autopsy 14 days after the final dose of gentamicin was found to be sterile on initial culture. Dilution of the homogenized preparations of renal tissue before culture, however, revealed that substantial numbers of *E. coli* had survived, in fact, and up to 2.5×10^5 *E. coli* could be demonstrated in the kidney in this way. An explanation for this observation was found when it was shown that bactericidal concentrations of gentamicin could be demonstrated in the renal cortex up to 14 days after the final administration. Renal cortical tissue was sterile in 88% of the tissue examined, but bacterial persistence was found in the medulla (29% sterile) where the concentration of gentamicin was comparatively low. Thus, a reciprocal relationship between the site of bacterial persistence and the concentration of antimicrobial agent in the kidney indicates how infection of the renal parenchyma can be maintained despite the presence of an apparent overall bactericidal concentration of gentamicin. These experiments demonstrate the importance of relating the pharmacokinetics of the antimicrobial agent and the bacteriologic features of the disease to local and anatomic characteristics of the kidney in the treatment of pyelonephritis.

Pharmacocinétique de la gentamycine dans le traitement de l'infection rénale. Un modèle de pyélonéphrite a été utilisé au cours d'expériences où la capacité de la gentamycine à éradiquer l'infection rénale a été étudiée. Des pyélonéphrites à *Escherichia coli* ont été induites chez 56 rats et le traitement par la gentamycine a été entrepris au 7ème jour et poursuivi pendant 11 jours. Le tissu rénal a été examiné à l'autopsie, 14 jours après la dernière administration de gentamycine, et il était stérile à la première culture. La dilution des préparations de tissu rénal homogénéisées avant la culture a révélé que *E. coli*, en nombre important, survit en fait et que jusqu'à $2{,}5 \times 10^5$ *E. coli* pouvaient être mis en évidence de cette façon. Une explication à cette constatation a été obtenue quand il a été montré que des concentrations bactéricides de gentamycine sont encore présentes dans le tissu rénal 14 jours après la cessation de l'administration. Le tissu rénal cortical était stérile dans 88% des tissus examinés mais la persistance bactérienne a été observée dans la médullaire (stérile dans 29% des échantillons) où la concentration de gentamycine est comparativement faible. Ainsi une relation réciproque entre le site de la persistance bactérienne et la concentration de l'agent antimicrobien dans le rein indique comment l'infection du parenchyme rénal peut se maintenir malgré la présence d'une concentration globale bactéricide de gentamycine. Ces expériences démontrent l'importance qu'il y a à relier la pharmacocinétique de l'agent antimicrobien et les caractéristiques bactériologiques de la maladie aux caractéristiques locales et anatomiques du rein dans le traitement de la pyélonéphrite.

Received for publication April 13, 1978
and in revised form July 7, 1978.

0085-2538/79/0015-0160 $01.40

The failure to eradicate renal infection by antimicrobial therapy is a problem commonly encountered in clinical practice. The fact that 25 to 50% of patients with urinary tract infection may have renal parenchymal infection and the frequency with which urinary tract infections recur [1–5] underscore the need for a continuing evaluation of the biologic basis of antimicrobial therapy.

An incidental observation in the course of recent experiments involving attempts to treat experimentally induced renal infection with gentamicin led us to suspect that despite apparently effective gentamicin treatment many viable microorganisms *(Escherichia coli)* remained in renal tissue. This was confirmed in further experiments where a reciprocal relationship between the persistence of infection in the renal cortex and medulla and the concentration of the antimicrobial agent in these tissues was shown. Thus, it has been possible to offer an explanation for the clinical observation of an appropriately selected and administered antimicrobial agent that has apparently failed to eradicate renal infection.

Methods

Experimental animals. Female animals weighing between 220 and 250 g were obtained from a random-bred strain of Wistar rat.

Experimental renal infection. Pyelonephritis was induced by the direct inoculation of *E. coli* 075 into

the surgically exposed kidney. Details of the method have been given previously [6].

Gentamicin administration. Gentamicin, 15 mg/kg, was injected i.m. daily for 11 days, beginning 1 week after the initiation of infection. Animals were killed 1, 4, 7, 11, 14, and 18 days after the termination of gentamicin treatment.

Sensitivity of the E. coli 075 to gentamicin. The minimum inhibitory and bactericidal concentration of gentamicin was determined to be 1.6 μg/ml.

Bacterial content of renal tissue. Kidneys were removed with a sterile procedure and homogenized in 5 ml of sterile saline. Nutrient agar pour plates were made from serial two-fold dilutions to obtain the bacterial count per gram of wet renal tissue. In reporting the bacteriologic data, the maximum number of *E. coli* found in the tissue samples was recorded at all times.

Tissue, blood, and urine preparation. Animals were killed in a carbon dioxide chamber, and the kidneys were removed immediately and decapsulated in sterile petri dishes. The medulla with attached papilla from each kidney was removed with a hollow cylindrical blade of 7-mm internal diameter. Cortical tissue on each side of the medullary cylinder was removed, and the crescent of cortex left after removal of the medullary plug was trimmed to remove residual medullary tissue. By this procedure it was possible to selectively dissect the entire kidney into cortical and medullary tissue. Tissue samples were weighed before being homogenized in 5 ml of sterile saline, and the homogenate was centrifuged to deposit tissue debris. The pH of the supernatant fluid was adjusted to a pH of 8.0 prior to analysis. Blood samples were collected either by venipuncture from a tail vein or by heart puncture at sacrifice. Samples of serum were analyzed immediately or stored at −70° C.

Antibiotic assays. The concentration of gentamicin in serum, urine, and tissue samples was determined by an agar-well-diffusion procedure as described by Bell and Wood [7]. Assays were carried out in duplicate with *Bacterium subtilis* as the indicator strain. The assay plate consisted of a 30-cm-square base of 6-mm-thick plate glass with a 50-mm-wide frame of the same glass on top. Difco antibiotic medium 11 agar (110 ml; pH, 8) containing 1 ml of *B. subtilis* was poured onto the warmed plate, a small amount seeping between the two plates to form a seal. Zones of inhibition by test samples were compared with a standard curve drawn from the zones of inhibition produced by seven known concentrations of gentamicin (0.06 to 5 μg/ml) prepared in a pH-8 buffer and diluted in normal serum. The diameters of the zones of inhibition were plotted on semilogarithmic paper, and the concentrations of antibiotics in the test samples were obtained directly from the standard curve. Serum concentrations were expressed as micrograms per milliliter, and the tissue concentration as micrograms per gram of tissue.

Methodologic controls. No antibacterial activity could be demonstrated when homogenates of kidney from untreated animals were examined. Possible binding of gentamicin to cortical and medullary tissue was also investigated. Recovery of gentamicin added to tissue homogenates was quantitative, thus failing to demonstrate binding within the range of concentrations encountered in these experiments, and no correction factor was necessary.

Results

Pyelonephritis. Injection of *E. coli* into the kidney with a glass microcapillary resulted in a consistent and reproducible infection in the renal parenchyma. The earliest gross pathologic changes were detected on the 4th day after infection. Lesions originated along the line of inoculation and extended from the cortex into the medulla of the kidney. Histologic evidence of active renal infection was detected 48 hr after the challenge. As the infection progressed, pitted and scarred areas characteristic of chronic pyelonephritis developed. (See Ref. 8 for photograph of gross changes.)

Apparent eradication of renal infection by gentamicin therapy. Experimental pyelonephritis was induced in a group of 56 animals which were subsequently treated with gentamicin for 11 days starting 7 days after challenge. The concentration of gentamicin in serum was determined at 30-min intervals over a 2.5-hr period after the administration of gentamicin, 15 mg/kg. Urine samples were obtained over a 24-hr period in a split collection made during the first 10-hr and the final 14-hr period, and the gentamicin concentrations of the samples were determined (Fig. 1). Thirty days after the initiation of infection the animals were killed, and the kidneys were removed for bacteriologic examination. Homogenates of renal tissue examined at autopsy 14 days after the final dose of gentamicin were found to be sterile on bacteriologic culture. Dilution of the homogenized preparations of renal tissue before incorporation into nutrient agar pour plates, however, revealed that a substantial number of viable *E. coli* had survived in the kidney. Of the 56 animals in this

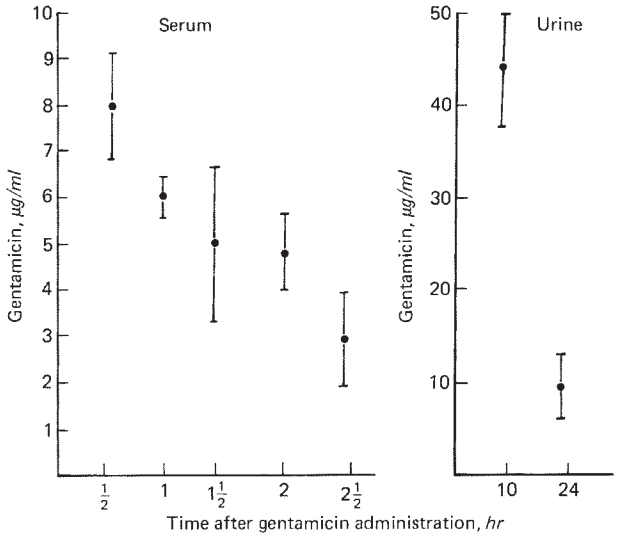

Fig. 1. *Serum and urinary concentrations of antibiotic attained after the i.m. administration of 15 mg/kg of gentamicin.* The figures for each time period are the mean ± 1 SD of the results obtained from 6 animals.

experiment that were challenged with *E. coli* 075 and treated with gentamicin, only 15 were in fact sterile when dilutions of homogenate were cultured. Of the remaining 41 animals, 37 showed a significant increase in microorganisms recoverable with dilution, and a maximum increase in bacterial numbers of $\log_{10}$ 1.1 to 5.4 (mean, 2.6) was found (Fig. 2). In control experiments with untreated animals, no increase in the bacterial content of kidney tissue was found when homogenates subjected to the same procedure were analyzed.

Does gentamicin accumulate in renal tissue in pyelonephritis? An inhibitory concentration of gentamicin in the pyelonephritic kidney was a likely explanation for the results observed. This possibility was investigated in an experiment where unilateral renal infection was induced in a group of 144 animals which were treated with gentamicin for 11 days starting 7 days after challenge. Animals were killed 1, 4, 7, 11, and 18 days after the cessation of treatment, and both left and right kidneys were removed. Gentamicin accumulation was demonstrable in all the kidneys examined up to 18 days after the cessation of treatment. Mean concentrations of 35 μg/ml were found during the first 4 days after the cessation of treatment, but bactericidal levels persisted in the tissue up to 18 days later. There were no significant differences in the concentration of gentamicin retained by the unmanipulated normal contralateral kidney and the pyelonephritic kidney (Fig. 3). When gentamicin concentrations in serum and urine were followed over the same period in a further group of 49 animals, gentamicin was barely detectable in the serum (<0.02 μg/ml) 24 hr after the final administration, whereas bactericidal concentrations of the antimicrobial agent could be demonstrated in the urine up to 7 days after cessation of therapy and were still detectable 18 days later (Fig. 4).

Does renal infection influence the intrarenal distribution of gentamicin? In the previous experiment, renal infection did not affect the accumulation of gentamicin by renal tissue. The fact that the lesion established involved a relatively small amount of renal tissue compared with the amount of normal tissue remaining may have obscured any difference. In the present experiment, the effect of renal infection on the intrarenal distribution of gentamicin was studied. A lesion was established in the upper pole of the kidney in 32 rats by the direct inoculation of *E. coli*. Gentamicin administration be-

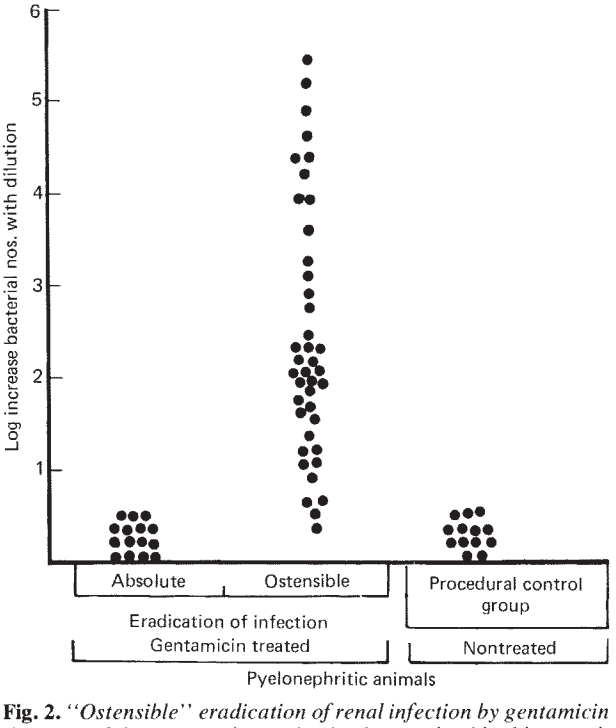

Fig. 2. *"Ostensible" eradication of renal infection by gentamicin therapy.* Of the 56 experimental animals examined in this experiment and found to be sterile on initial examination, only 15 (absolute eradication) remained sterile when dilutions of the kidney homogenate were cultured. In 37 of the remaining 41 animals, eradication of infection was "ostensible" in that up to a 10,000-fold increase in the number of microorganisms in the kidney were demonstrable when homogenized preparations of renal tissue were diluted before bacteriologic culture.

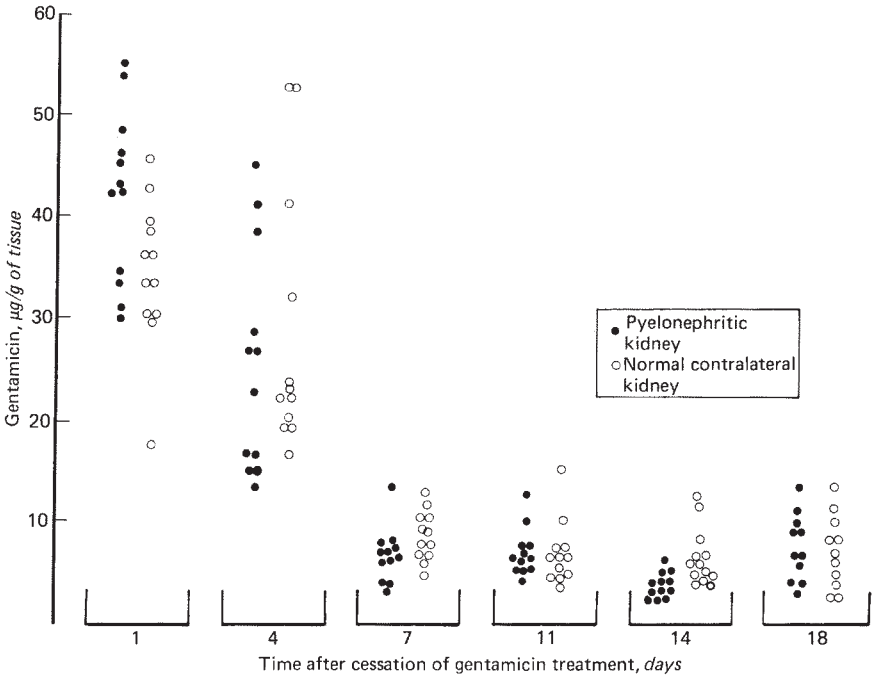

Fig. 3. *Effect of pyelonephritis on the concentration of gentamicin in renal tissue.* Animals with unilateral pyelonephritis were killed from 1 to 18 days after the termination of an 11-day course of gentamicin (15 mg/kg/day), and the concentrations of gentamicin in the pyelonephritic and unmanipulated normal contralateral kidneys were determined.

gan 7 days after challenge and was continued for 11 days. Animals were killed 1, 4, 11, and 18 days after the final day of gentamicin treatment, and the kidneys were removed and bisected to separate the upper and lower poles. The renal lesions, however, did not influence the distribution of gentamicin, and similar concentrations of the antimicrobial agent were found in both poles of the kidney from 1 to 18 days after the cessation of treatment (Fig. 5), although it should be appreciated that the methodology may not be sufficiently sensitive to detect changes in neighboring areas in the vicinity of infected tissue. In an additional experiment, the gross pathologic and histopathologic features of the lesions in the kidneys of animals challenged in the upper pole of one kidney were determined (Fig. 6).

Site of gentamicin and E. coli localization within the kidney. Local sequestration resulting in a gradient of gentamicin within the kidney could explain the ostensible eradication of infection reported in Fig. 2. Renal infection was induced in two groups of animals, both of which were treated with gentamicin (15 mg/kg) for 11 days starting 1 week after challenge. The two groups were killed 7 and 11 days after cessation of treatment, and the concentration of gentamicin and the number of residual microorganisms in the renal cortex and medulla were determined. The concentration of gentamicin in the renal cortex in animals examined 7 days after the cessation of therapy was significantly higher than it was in medullary tissue from the same animals ($P < 0.01$, by Wilcoxon sum of ranks analysis; Fig. 7). Renal infection had been eradicated from cortical tissue in 21 out of 24 of the animals challenged (88%), whereas medullary tissue was found to be sterile in only 7 out of 24 cases (29%). Similar results were obtained when renal tissue was examined 11 days after the cessation of therapy.

Discussion

Our biologic experiments and the observation that eradication of renal infection following gentamicin therapy was apparent rather than real led us to conclude that the reciprocal relationship between the site of bacterial persistence and the concentration of antimicrobial agent in the kidney explained how infection of the renal parenchyma could be maintained despite the presence of apparently bactericidal concentrations of gentamicin.

Although antibiotics show remarkable variations in their ability to concentrate in different tissues, the accumulation and persistence of the amino-

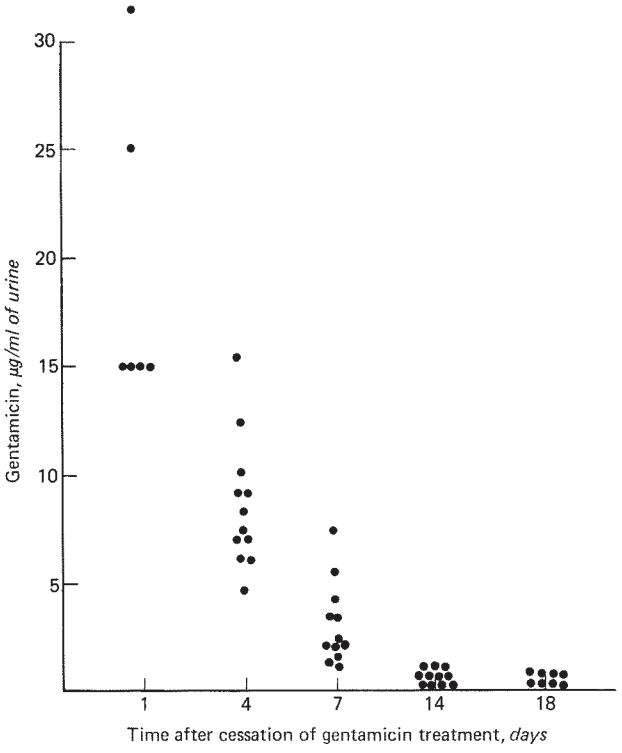

Fig. 4. *Urinary excretion of gentamicin after the termination of antimicrobial therapy.* Bactericidal levels of the antimicrobial agent could be demonstrated in the urine up to 7 days after cessation of therapy and were still detectable 18 days later.

glycosides by renal tissue appear to be unique. The suggestion that gentamicin may accumulate in the kidney was first reported in 1974 by Whelton and Walker [9] and was commented on by Kahlmeter and Kamme [10] in a letter to *Lancet* claiming that they were able to detect the antibiotic in urine 20 days after therapy had been discontinued. Wahlig [11] later confirmed this observation experimentally by showing that the bulk of the antibiotic was excreted in the urine within 24 hours but slow excretion continued for several days after serum concentrations had become undetectable. These findings have been confirmed and extended by the direct examination of gentamicin levels in the tissue of experimental animals [12, 13] and in the genitourinary tissue in man [14] during studies of the pharmacokinetics of gentamicin. An important feature of our experiments has been the disclosure of a reciprocal relationship between the site of bacterial persistence and the concentration of antimicrobial agent in the kidney which has provided an explanation for the persistence of renal infection, despite seemingly adequate concentrations of gentamicin in serum, urine, and renal tissue. The concept of a reciprocal relationship between bacterial persistence and antimicrobial concentration in cortical and medullary tissue arose from our observations of the distribution of gentamicin and microorganisms in renal tissue after therapy had been discontinued. The distribution of gentamicin within the kidney during therapy was not determined in the present experiments; however, the pharmacokinetics found by us during the posttherapy period are similar to those observed by Fabre et al [13] in the period immediately following the administration of gentamicin, and it is reasonable to assume that a marked cortical-medullary gradient was also present in our animals during gentamicin administration. The experiments have shown that bactericidal concentrations of gentamicin were still present in the renal cortex at least 14 days after therapy had been discontinued, and as a result of the high concentration of gentamicin, cortical tissue was usually sterile. Bacterial persistence, however, was found in the medulla where the concentration of the antimicrobial agent was comparatively low. The relationship between the site of bacterial persistence and antimicrobial concentration was not absolute,

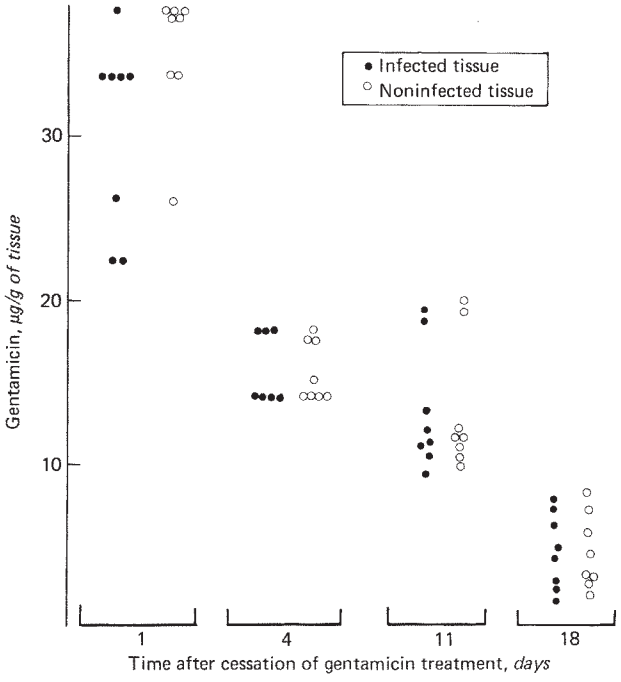

Fig. 5. *Effect of pyelonephritis on the intrarenal distribution of gentamicin.* Pyelonephritis was established in the upper pole of individual kidneys, and the animals were killed 1 to 18 days after the cessation of gentamicin treatment.

but this was to be expected. The procedure for the division of the kidney into cortical and medullary tissue was essentially a macroscopic dissection, and some admixture of the pericortical zone of cortical and medullary tissue would have occurred with individual dissections. Nevertheless 88% of cortical tissue sectioned was sterile, whereas infection was eradicated in only 29% of the medullary tissue examined. The other major contribution of the present study has been to determine the effect of a renal infection on the ability of gentamicin to reach therapeutically effective levels in the pyelonephritic kidney. With an experimental model, the concentration of gentamicin was shown to be similar in both the pyelonephritic and the contralateral unchallenged kidney, and similar results were obtained when the gentamicin content of normal and pathologically altered tissue within the pyelonephritic kidney was studied. The procedures used in these analyses, however, were not sufficiently sensitive to exclude the possibility that in neighboring areas within the local environment of an infected site, the concentration of gentamicin would not be affected by pathologic damage to the kidney. Further studies using autoradiographic procedures to localize radioactively labeled gentamicin will be needed to resolve this question.

The biologic significance of the renal accumulation of gentamicin has not been widely recognized, although Whelton and others have attempted to determine the therapeutic implications in severe renal disease [15]. Chronic diseases in the kidney may present a significant barrier to the penetration of some antibiotics into the kidney, and in the case of gentamicin, substantially reduced concentrations have been found in diseased kidneys. Most of the available information, however, has been derived from studies of human kidneys with end-stage renal disease unrelated to the pathologic changes found in renal infection [16] and the present experiments demonstrate the ability of a relevant experimental model to provide information on the pharmacokinetics of gentamicin in a localized infectious lesion. The cellular or subcellular site of gentamicin accumulation has not been determined, although the binding appears to be associated with the particulate cell fractions [17]. The changes described by

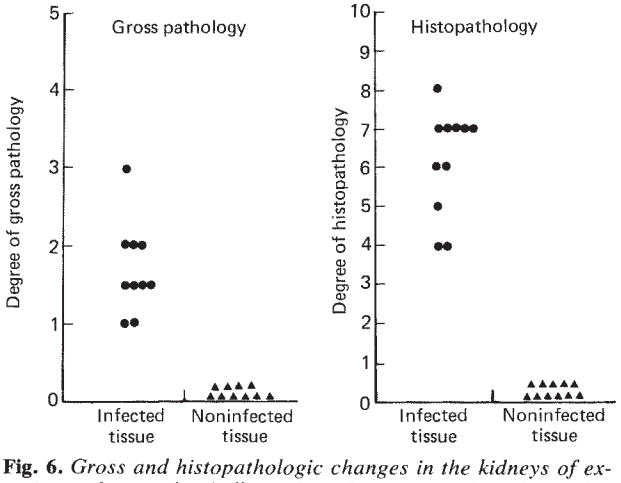

Fig. 6. *Gross and histopathologic changes in the kidneys of experimental animals challenged in the upper pole only.* Quantitation of the gross pathology was made on a 0 to 10 scale, with each unit of the scale representing one tenth of the surface of the kidney. In the case of histopathology, 10 represented lesions involving 25% or more of the area of the section. Lesser degrees of damage were scored on a 0 to 10 scale, where each unit represented 2.5% of the area of the kidney.

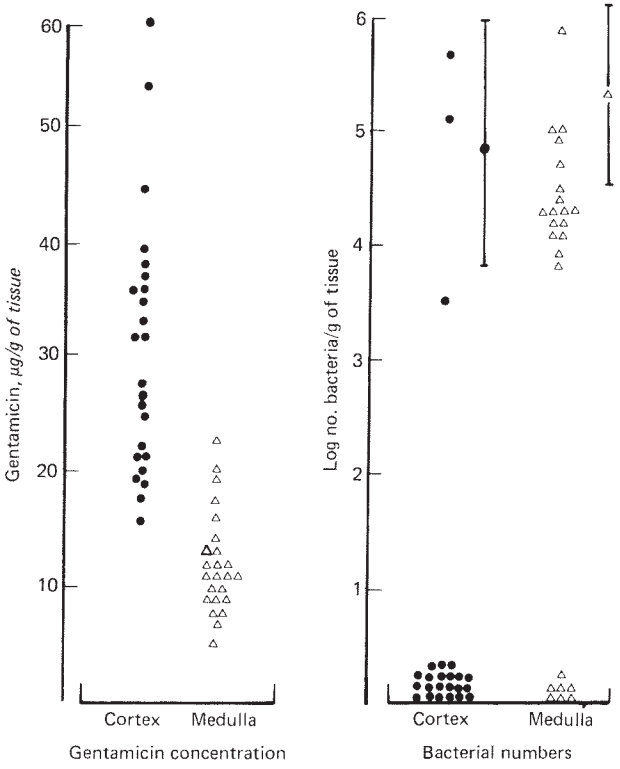

Fig. 7. *Localization of gentamicin and E. coli within the kidney.* Renal infection was induced in two groups of animals which were treated with gentamicin and killed 7 days after treatment had ceased. Quantitative analysis of the distribution of *E. coli* and gentamicin in the renal cortex and medulla shows a reciprocal relationship between the concentration of antimicrobial agent in the kidney and the site of bacterial persistence. The *vertical bars* in the right-hand frame of the figure represent the full range of bacterial counts in the cortex and medulla of 12 untreated animals challenged and examined at the same time as the experimental group.

Patel et al [18] and Kosek et al [19], who used the electron microscope to show cytosegrosomes with myeloid bodies within the proximal tubular cells, may be of significance, but the association between renal cortical aminoglycoside accumulation and cytosegrosome formation must await the precise intracellular location of the aminoglycoside antibiotics.

The demonstration that organisms may persist in renal tissue despite apparently bactericidal levels of antimicrobial agent is an observation of some years standing [20]. The most logical explanation is that the concentrations of antimicrobial agent determined quantitatively in blood, tissue, and urine samples, which in the present experiments were 5, 25, and 28 times the minimum bactericidal concentration of the infecting organism, do not approximate the concentration of gentamicin actually achieved at the site of microbial sequestration. Our interpretation of the relationship between cortical and medullary concentrations of gentamicin and the bacteriologic status of the kidney is that the concentration of antimicrobial agent present in these anatomical sites, when determined by current procedures, represents only the comparative potential of the antibiotic to achieve a bactericidal concentration at the site of infection. The simple achievement of a bactericidal tissue concentration of an antimicrobial agent, even when favorably related to the minimum bactericidal concentration of the infecting organism, is not an accurate predictor of the ability of an antimicrobial agent to eradicate a local renal infection.

In summary, a study of the gradient patterns of gentamicin and microorganisms following experimentally induced and treated renal infection has provided an explanation for the observation that infection in the kidney may persist despite adequate therapy with appropriately selected antimicrobial agents. The experiments have emphasized the importance of considering the anatomic, pharmacologic, and bacteriologic features of the local environment in the management of renal infection.

Acknowledgments

This study was supported by the Medical Research Council of New Zealand. The Department of Microbiology, Auckland Hospital, assisted in establishing the assay for gentamicin. The pharmaceutical preparation of gentamicin used in these experiments was generously provided by the Schering Corporation.

Reprint requests to Dr. T. Miller, Department of Medicine, Auckland Hospital, Park Road, Auckland, New Zealand

References

1. Stamey TA, Govan DE, Palmer JM: The location and treatment of urinary tract infections: the role of bactericidal urine levels as opposed to serum levels. *Medicine (Baltimore)* 44:1-36, 1965
2. Reeves DS, Brumfitt W: Location of urinary tract infection: a comparative study of methods, in *Urinary Tract Infection*, edited by O'Grady F, Brumfitt W, London, Oxford University Press, 1968, pp. 53-67
3. Fairley KF, Bond AG, Brown RB: Simple test to determine the site of urinary-tract infection. *Lancet* 2:427-428, 1967
4. Fairley KF: Localization of urinary-tract infection. *Lancet* 1:1212, 1969
5. Ronald AR, Cutler RE, Turck M: Effect of bacteriuria on renal concentrating mechanisms. *Ann intern med* 70:723-733, 1969
6. Miller TE, Robinson KB: Experimental pyelonephritis: a new method for inducing pyelonephritis in the rat. *J Infect Dis* 127:307-310, 1973
7. Bell SM, Wood R: An antibiotic assay method. *J Med Lab Technol* 25:27-32, 1968
8. Miller TE, North D, Burnham S: Acquiescent renal infection. *Kidney Int* 7:413-421, 1975
9. Whelton A, Walker WG: Intrarenal antibiotic distribution in health and disease. *Kidney Int* 6:131-137, 1974
10. Kahlmeter G, Kamme C: Prolonged excretion of gentamicin in a patient with unimpaired renal function. *Lancet* 1:286, 1975
11. Wahlig H: Animal studies on tissue concentrations of gentamicin, in *Proceedings of the Eighth International Congress on Chemotherapy*, Athens, Greece, 1973, abst. A-74
12. Luft FC, Kleit SA: Renal parenchymal accumulation of aminoglycoside antibiotics in rats. *J Infect Dis* 130:656- 659, 1974
13. Fabre J, Rudhardt M, Blanchard P, Regamey C: Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. *Kidney Int* 10:444-449, 1976
14. Alfthan O, Renkonen OV, Sivonen A: Concentration of gentamicin in serum, urine and urogenital tissue in man. *Acta Pathol Microbiol Scand [B]* 81 (suppl 241):92-94, 1973
15. Whelton A, Carter GC, Bryant HH, Fox L, Walker WG: Therapeutic implications of gentamicin accumulation in severely diseased kidneys. *Arch Intern Med* 136:172-176, 1976
16. Luft FC, Yum MN, Walker PD, Kleit SA: Gentamicin gradient patterns and morphological changes in human kidneys. *Nephron* 18:167-174, 1977
17. Kunin CM: Binding of antibiotics to tissue homogenates. *J Infect Dis* 121:55-64, 1970
18. Patel V, Luft FC, Yum MN, Patel B, Zeman W, Kleit SA: Enzymuria in gentamicin-induced kidney damage. *Antimicrob Agents Chemother* 7:364-369, 1975
19. Kosek JC, Mazze RI, Cousins MJ:Nephrotoxicity of gentamicin. *Lab Invest* 30:48-57, 1974
20. Lipman RL, Tyrell E, Small J, Shapiro AP: Evaluation of antibiotic therapy in acute pyelonephritis produced by Escherichia coli in rats. *J Lab Clin Med* 67:546-558, 1966